Biology and Behavior of the South American Moth, *Cyanotricha necyria* (Felder and Rogenhofer) (Lepidoptera: Notodontidae), a Potential Biocontrol Agent in Hawaii of the Forest Weed, *Passiflora mollissima* (HBK) Bailey

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ABSTRACT

The life cycle of the moth *Cyanotricha necyria* was studied at ambient outdoor temperatures in a quarantine facility at 1,140 m (3,800 ft.) on the island of Hawaii to determine if the insect would be a suitable biological control agent for the forest weed *Passiflora mollissima*. This moth deposits eggs in clusters on the underside of 2- to 4-week-old leaves; first-instar larvae feed gregariously at the leaf margin, but second instars disperse and become solitary. The larvae pass through four instars before pupation. The cocoon is a thin, semi-transparent, net-like structure and is spun in a crevice or enclosed in a folded leaf. At ambient outdoor temperatures, total development from egg to adult required 90 days in summer and 120 days in winter. Mating occurred between 5 and 10 days after females emerged, and maximum egg production was achieved between 10 and 25 days. Females lived an average of 32 days with a few surviving, and laying eggs, for up to 45 days. Egg production in breeding colonies in the laboratory averaged 3.7 eggs per female per day with an average female laying a total of 67 eggs. However, in both laboratory and field collected eggs, fertility averaged between 45 and 55%. Among field-collected larvae and pupae, 10 to 100% were parasitized by three species of Hymenoptera.

Defoliation of *Passiflora mollissima* (Humboldt, Bonpland and Kunth) Bailey, by the larvae of the moth *Cyanotricha necyria* (Felder and Rogenhofer) (Posada et al. 1976) is an occasional problem in commercial fields of this plant (Castanaeda 1956, Martin and Nakasone 1970) at elevations of 2,100 to 3,000 m (7,000 to 10,000 ft.) in the Andes Mountains of Colombia, Ecuador and Peru. While larvae of the moth are present in most fields, they generally cause minor damage. We have occasionally observed outbreaks that completely defoliated small local plantings. In Hawaii, *P. mollissima*, commonly referred to as banana poka (La Rosa 1985), was probably introduced as an ornamental, but has escaped cultivation and invaded native forests where it is now considered the major threat to their continued existence (La Rosa 1984, Waage et al. 1981, Warshauer et al. 1983). In an effort to control this weed, *C. necyria* was tested as a potential biocontrol agent, and approved for release by the Hawaii Department of Agriculture. The

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first field release was made above Laupahoehoe on the Island of Hawaii on February 3, 1988.

Six shipments of insects, collected in Colombia and Ecuador between 1984 and 1986, were sent to Hawaii for evaluation. Methods were developed for propagating *C. necyria* and laboratory colonies were established. These colonies were used primarily for host-testing studies to determine the suitability of this insect for release in Hawaii. However, during the development of propagation methods, and by incidental observations, the life cycle under laboratory conditions was determined. This paper reports some details of the life cycle of *C. necyria* from studies at Hawaii Volcanoes National Park Quarantine Facility (HVNPQF) Hawaii.

MATERIALS AND METHODS

The Park's Quarantine Facility is located in a native koa-ohia-tree fern rainforest (2,250 mm precipitation) at 1,140 m (3,800 ft.) elevation. The locality closely approximated the preferred climatic habitat of *P. mollissima* (Warshauer et al. 1983), although the nearest infestation of this weed was 3 km away. The Facility was maintained at near ambient temperature (average January temperature 16°C, August 20.5°C) and humidity by circulation of filtered outdoor air. Insects were reared in standard sleeve cages (45 cm × 45 cm × 50 cm tall) or in perforated plastic sacks containing bouquets of freshly picked *P. mollissima* foliage. Colonies used in the study originated from the Pasto and Ipialis areas of Colombia near the Ecuador border. Most of our observations were made on either field-collected insects shipped to the Quarantine, or on the first or second laboratory generations.

OBSERVATIONS

Adults. *C. necyria* is a medium-sized, heavy-bodied moth with a wing span range of 3 to 3.5 cm. Males and females are identically colored an iridescent blue-black, with an elongated orange spot located near the base of the anterior edge of the forewing. Sexes can be separated by the antennae which in the males are feather-like (Fig. 1). The sex ratio of adults reared from field-collected pupae, and from larvae reared in the laboratory, was 1:1.

Emergence from the pupa usually occurred in the morning while temperatures were increasing. Wing expansion occurred within half an hour and the meconium was passed between 1 and 12 hours after emergence.

The adult moth has a well developed proboscis (~1/5 cm long) which is used for feeding on nectar from small flowers with corollas less than 1 cm in length. However, the proboscis is too short to feed on nectar of the flowers of *P. mollissima*, the corolla of which is over 6 cm long. In the laboratory, adults readily fed on honey streaked on the glass top of the sleeve cages, wicks moistened with sugar water or sweetened fruit juice, Homoptera honeydew, and the secretions of extrafloral nectaries on margins of the *P. mollissima* leaves. Various protein sources (fermented fruit,

yeasts, meats, animal scats and protein additives) were offered, but adults showed no interest in these foods. Extrafloral nectary secretions of *Passiflora* are known to contain amino acids (Durkee 1982) that can serve as precursors for protein synthesis.

Premating activity was not observed, but the large antenna of the males suggest that in the field, females produce a sex pheromone. In laboratory sleeve cages, females mated between 5 and 10 days after emergence. Mating was generally noticed in the morning while the temperature was rising and took place either on the upper surface of the leaves or the vertical sides of the cages.

Females were not sexually mature on emergence and, on dissection, were found to contain only small undeveloped eggs and to have soft genitalia. After 5 days, the first eggs were full-sized with a well developed chorion, and the genitalia had hardened. Eggs may be laid before mating since the first few eggs usually were scattered singly about the plant and were infertile. However, after 8 to 10 days, fertile eggs were laid in clusters. Adult life span of the male is ~30 days. The adult female life span was ~35 days, although individual females lived as long as 45 days, with peak egg laying occurring about 15 days after emergence. Twenty-five females that died after 25 days were dissected. All but one contained some fully developed eggs (5 to 15) ready to be laid, and at least 50 to 100 more eggs in all stages of development. This was interpreted as indicating that, in the laboratory, egg laying was terminated early by the premature death of the female, due either to confinement or to the lack of an appropriate nutrient.

Eggs. Eggs were spherical, weighed about 0.00035 gm, were about 0.9 mm in diameter, and lacked obvious sculpturing (Fig. 2). When freshly laid, eggs were light-blue to pale white and were attached by an adhesive at the base to the underlying substrate, but not to adjacent eggs in the same egg masses.

Fifty shoots of *P. mollissima* that had been placed in breeding cages were examined for eggs. Of the eggs found, 84% (1,241 +) were laid on the underside of leaves, 14% were found on the upper surface of the leaf and 2% on the stems, flower buds, or glass tops or walls of the sleeve cages. Females also showed an age preference in selecting leaves for oviposition. Eggs were rarely laid on the expanding new leaves at the shoot tip. Most oviposition occurred on leaves between 8 to 12 cm long with a hard, waxy upper surface. These were located between 5 and 8 leaves (15 to 40 cm) back from the growing tip and were 2 to 4 weeks old. Leaves older than 4 weeks were seldom used.

Eggs generally were laid adjacent to each other in compact, single-layered clusters. In the laboratory, 25 isolated females produced clusters averaging 6.8 eggs (range 1 to 14 eggs). Occasionally, in the field or in large breeding colonies, more than one female would combine in laying larger clusters which contained up to 31 eggs. Scattered single eggs were also laid at random over the foliage of the plant, or on cage walls or top. In general, these single, scattered eggs were not fertile. Isolated females 10 to 20 days old laid an average of 9.4 eggs/day. However, in the large breeding colonies

we estimated that half the females were unmated, and production per female was only about 3.4 eggs per day, with a mean lifetime production of 64.6 (range 0 to 184.4).

In the laboratory, egg fertility was low, averaging 54.6%. Low fertility originally was assumed to be the result of poor mating or inadequate nutrition under laboratory conditions. In 1986, a field collection of 96 egg masses from near Pasto, Colombia, was shipped to the HVNPQF, but only 46% of the eggs were fertile. Therefore, low fertility may also occur in the field.

Egg development was temperature-dependent and at constant temperatures, occurred from 10 to 25°C (requiring 25 to 11 days). At outdoor ambient temperatures eggs required between 18 and 21 days to hatch, depending on the season of the year. The first signs of development were generally seen at ~12 days when the black eyes and mandible tips of the larvae became apparent. Eggs continued to darken until 2 or 3 days before hatching, when the fully developed larva could be seen through the clear chorion.

Larvae. At eclosion, first-instar larvae were ~3 mm long, grey in color with a black head. Mean weight at hatching was 0.3 mg (Fig. 2). Within 3 to 5 days they became black with yellow longitudinal stripes and a solid black head. The larvae ate half of the egg chorion at emergence, then, with siblings from the same egg mass, migrated to the margin of the leaf and began feeding gregariously. The larvae remained gregarious through the first and part of the second stadium, but when ~1 cm in length they began to disperse, and through the remainder of their development were generally solitary feeders. There were four larval instars (Table 1). Fully mature larvae were ~2.6 cm long and weighed ~180 mg. They were black with longitudinal yellow stripes, and scattered tuffs of fine, short, white setae (Fig. 3).

TABLE 1. Mean and SD of the Four Instars of *C. necyria* Larvae Reared at 15°C.

		1st	2nd	3rd	4th
Head Capsule Width (mm)[1]	$\bar{x}$	0.58	0.89	1.32	1.98
	SD	±0.04	±0.06	±0.10	±0.20
	Range	0.51 to 0.69	0.76 to 1.06	1.10 to 1.53	1.61 to 2.30
	N	67	116	111	188
Length (mm), at End of Instar (N = 15)	$\bar{x}$	5.00	11.60	18.27	25.80
	SD	±1.13	±1.96	±2.37	±1.37
Weight of Larvae (mg), at End of Instar	$\bar{x}$	2.50[2]	11.50	42.00	180.40
	SD	±0.10	±0.80	±3.00	±4.40
	N	10	10	10	20
Development Time Days (N = 15)	$\bar{x}$	10.10	23.87	14.70	11.70
	SD	±4.90	±6.53	±3.60	±3.70

[1]Head-capsule measurements were from preserved specimens; all other measurements from live larvae.
[2]Mean weight at eclosion, 0.3 mg.

Molting between instars required several days. The larva left the feeding site and moved several centimeters or more, to an area away from other larvae. A fine oval-shaped silkened pad, slightly larger than the length of the larva, was spun on the underside of a leaf. Proleg crochets were firmly anchored into the pad and the larva remained there during the 2 to 3 days required for molting. Once the old skin was shed, the callow form required 2 to 3 hours to harden and regain full coloration.

First-instar larvae began feeding on the leaf on which the egg mass was deposited, which by then was about 1 to 1.5 mo. old. As dispersal began, larvae usually continued feeding on older leaves and ignored the soft, expanding leaves at the tip of the shoot, unless all other leaves had been consumed. Under conditions of total defoliation, large larvae would also consume leaf tips, tendrals, flower buds, flowers and even attempt to feed on green fruit. During its development, a larva consumed about 36.3 cm^2 of leaf, with an estimated fresh weight of 0.89 gm. An average 1-month-old leaf had an area of about 50.6 cm^2. Therefore, during its development a larva consumed less than a single leaf. However, feeding was not restricted to a single leaf, and most larvae apparently changed leaves at least 3 times.

At constant temperatures, larval development to pupation required from 26 days at 25°C to 83 days at 10°C. Larval development required 35 to 45 days at ambient outdoor air temperature at the HVNPQF, depending on the season.

C. necyria larvae showed none of the defense mechanisms typical of most other Lepidoptera larvae. They fed on the underside of the leaf during all four stadia in an exposed position that offered minimal protection. Caterpillar coloration, black with vivid yellow stripes, was conspicuous against the green leaves. When larvae were prodded they simply gripped the leaf more tightly and reared both head and tail into the air and remained motionless. If larvae were prodded more vigorously, a light green, fleshy tubercle (Fig. 4) was projected from the ventral surface of the prothorax. This was apparently the outlet of a gland. Most genera of North American Notodontidae have a gland at this location which is used to spray a defensive secretion such as formic acid (Godfrey and Appleby 1987). *C. necyria* larvae were not observed to spray a liquid with this structure, but did secrete a small droplet of green liquid. It was suspected that this liquid was an irritant or repellent that may play a role in the larval defense. Another defense mechanism may be the silk pad on which the larvae rested during molting. Since larvae were inactive and susceptible to predation at this stage, we suspected that this pad may create a barrier which prevents predators from reaching the larva.

Spinning and Pupation. Upon completion of feeding, mature larvae left the feeding site and migrated to high points on the plant (or in the laboratory, to the roof and top of the sleeve cages) to pupate. In the laboratory, the cocoon was usually spun in the upper corner of the cage where the walls and roof formed two sides of the cocoon. When pupation occurred on the plant, the larva folded a leaf, sealed the edges with silk and constructed a silk barrier across either end of this partial tube. In the laboratory, the

cocoon required 2 to 3 days to complete and was ~2 cm long and ~1 cm in diameter. Open surfaces of the cocoon were constructed of a flimsy, net-like weave, thin enough that the prepupa or pupa inside could be easily observed. Inside the cocoon, the pupa did not rest free, but was suspended at its center by a network of threads. After completing the cocoon, the larva remained quiescent in a prepupal stage for 3 to 5 days before pupating. Cocoon construction and prepupal stage required 5 to 10 days, depending upon the temperature. The new pupa was light-green, but hardened in 24 hours and became dark-brown with small, orange spots on the abdomen. By 10 to 15 days, the pupa was solid black. The pupa was ~1.5 cm long, weighed ~100 mg and had a smooth exterior except for a cluster of crochets on the end of the cremaster. At outside ambient air temperatures, the pupal stage lasted from 30 days in summer to 45 days in winter. Pupae also were held at 7°C for up to 35 days, and, when removed, resumed normal development.

Parasites and Predators. In rearing ~1,700 field-collected eggs from Pasto and Ipialis areas of Colombia, we found no egg parasites. However, 10 to 100% of field-collected pupae and late instar larvae shipped to the HVNPQF were parasitized by two species of Ichneumonidae, *Coccygomimus pepsoides* Porter and *Ichneumon* sp., and by a Torymidae, *Perissocentrus* sp. Larval parasitism was probably a major mortality agent in the field.

Predation was not studied directly, but occasionally occurred when predators were inadvertently brought in on bouquets of field-collected *P. mollissima* foliage and introduced into rearing cages. When found, predators were separated into petri dishes and fed surplus *C. necyria* larvae for further observations. None of the predators encountered on field-collected *P. mollissima* foliage in Hawaii was large enough to attack third- or fourth-instar larvae. However, the predaceous larvae of a green lacewing were encountered fairly frequently. These readily attacked first-instar larvae, and consumed over 20 larvae before pupating. Similarly several small wolf spiders attacked first- and second-instar larvae. From these limited observations, we concluded that *C. necyria* larvae, at least in the first two instars, probably would suffer some mortality from naturally-occurring predators in Hawaii.

Pathogens. The most important mortality agent identified to date is a pathogen. New larvae shipped from the field were placed individually in petri dishes to isolate those that might contain pathogens. When reared in this manner, all shipments suffered between 10 and 40% mortality that was considered disease-related. A few of the diseases were identified as fungi or bacteria by microscopic examination. However, in most cases no pathogen was identified. Originally it was suspected that a virus was present. To eliminate any possible virus, first generation eggs were washed in a 1% clorox solution and the resulting larvae again propagated individually through the first laboratory generation. We believed that by the second generation, disease had been eliminated. Larval survival to pupation then averaged over 70%. Larvae were propagated by placing 100 in individual cages with bouquets of foliage. This procedure gave satisfactory rearing

through the second and third generations with survival ranging between 70 and 90%. However, by the fourth generation, mortality was often 50%, and by the fifth generation increased to greater than 95%.

Specimens submitted to two insect pathologists (Dr. Mauro Martignomi, USDA Forest Service, Corvallis, Oregon; and Dr. Minoru Tamashiro, Department of Entomology, University of Hawaii-Manoa) were identified as having been killed by microsporidia. Unlike most insect pathogens, microsporidia often do not kill their host outright, but can be propagated for several generations at sublethal levels within the host population. This apparently happened in our colonies when adults passed the microsporidia spores to the next generation inside the clorox-treated eggs. This was confirmed by dissection of all stages of the insect from an infected colony. Microsporidia spores were found in adult females, pupae, all stages of larvae, as well as in both fertile and unfertilized eggs. Once identified, the disease was controlled by dipping the *P. mollissima* leaves used to feed the larvae in a solution of the fungicide Benomyl (Hsiao and Hsiao 1973). Rearing larvae on Benomyl-treated foliage not only immediately stopped the progress of the disease, but by the next generation had usually eliminated all signs of it. This disease is now suspected of having destroyed several early colonies that originated at different locations or times in Colombia and Ecuador. It has been identified in most subsequent collections from South America. We do not know how common or virulent the pathogen is in South America, or whether it plays any role in control of wild populations of *C. necyria*.

Climatic Range. In South America, we observed *C. necyria* only on the cool, wet slopes of the Andes between 2,100 and 3,000 m. Nighttime temperatures there ranged from 5 to 10°C and daytime temperatures mostly from 15 to 20°C, up to 25°C in direct sunlight. Under quarantine in Hawaii, adult activity began at 15°, flying between 20 and 25°C, mating from 15 to 25°C and egg laying from 15 to 25°C. Above 25°C, adults became totally immobile and hid under leaves.

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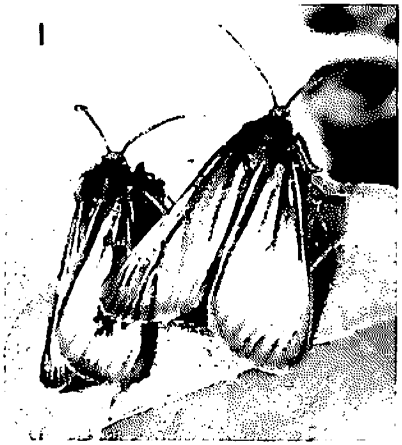

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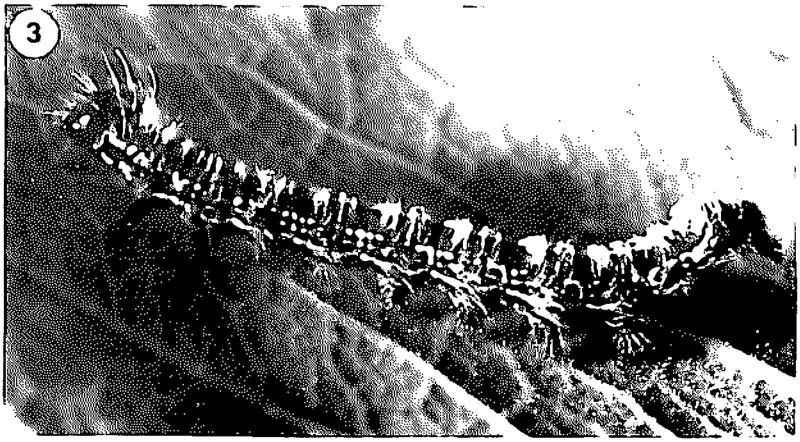

FIGURE 1. Pair of adult *C. necyria*, male is on left with feather-like antenna.

FIGURE 2. Larvae of *C. necyria* at eclosion (larval length approx. 3 mm), the two solid color eggs at left are infertile.

FIGURE 3. Late fourth instar *C. necyria* larva just before starting cocoon. Larva is approx. 2.5 cm long.

FIGURE 4. The extruded orifice of a gland on the ventral surface of the prothorax is visible just behind head of this fourth instar larvae of *C. necyria*. Function of this gland is not known.